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A Portable, Discrete-Sampling Submersible Plankton Pump and Its Use in Sampling Starfish Eggs

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Abstract. The apparatus was designed to enable divers to obtain discrete replicate samples from known volumes of water. Relatively large volumes of water can be filtered without the need to maintain contact with or return to the surface. These attributes confer a high degree of flexibility in subsurface sampling situations. Although we used the equipment to sample the eggs of invertebrates, it could equally easily be used to sample other small, patchily distributed plankton.

Introduction

In the marine environment, just as in the terrestrial environment, many phenomena of interest to ecologists vary unpredictably in time and space. However, the nature of aquatic environments greatly complicates our attempts to understand these ecosystems. The advent of scuba diving has transformed our understanding of life in marine and fresh waters, through the freedom it has given scientists to explore them directly. Many aspects of marine ecological research continue to present difficulties, particularly when sampling programs must be conducted at fine spatial or temporal scales in a patchy environment.

In a study of *in situ* fertilization rates of the crown-of-thorns starfish, *Acanthaster planci*, we needed to sample eggs repeatedly from positions that were fixed relative to spawning starfish but that were unpredictable in time and space. Sampling had to be conducted both during experimentally induced spawning situations and during spontaneous natural spawning events. The sampling method had to enable us to sample and filter measured quantities of water without damaging starfish eggs, and the equipment had to be easily operated and transported by a scuba diver. We designed and constructed an apparatus that enabled us to fulfill these requirements and obtain data on fertilization rates of *A. planci* and other echinoderms in both natural and experimental situations.

In applying this apparatus to the study of *in situ* fertilization rates, it was important to determine that estimates of fertilization rates were not biased by the sampling procedure or the apparatus. This paper describes the apparatus, the sampling procedure, and the experiments investigating potential sources of bias.

Materials and Methods

Apparatus

Conceptually, the apparatus was a metered plankton pump with 12 replicate filter cartridges (Fig. 1a). Its dimensions were 500 × 400 mm and 550 mm high. Water to be sampled was taken in through a suction hose (13-mm Tygon tubing) and passed through a digital flow meter (GPI electronic digital meter) after filtration. Suction was applied using a pump (Flojet, 12 l/min) attached to the filter cartridge by a second hose. Each numbered filter cartridge consisted of an inner filter tube constructed of clear acrylic and plankton mesh (60 μm), approximately 350 ml in volume, surrounded by a second outer tube of 500-ml volume that screwed into a flange at the top of the inner tube (Fig. 1b). The entire cartridge was then screwed onto the pump by another set of threads on the flange. Water was drawn into the cartridge through a one-way fitting mounted in the pump assembly at the top of the inner tube, and out of the cartridge through a second one-way fitting at the bottom of the outer tube. Thus once the sample was drawn into the tube, it was effectively isolated from the surrounding water mass. As successive samples were taken, the suction hose and the second hose to the pump were removed and reattached to the appropriate cartridge. The flow meter was activated automati-

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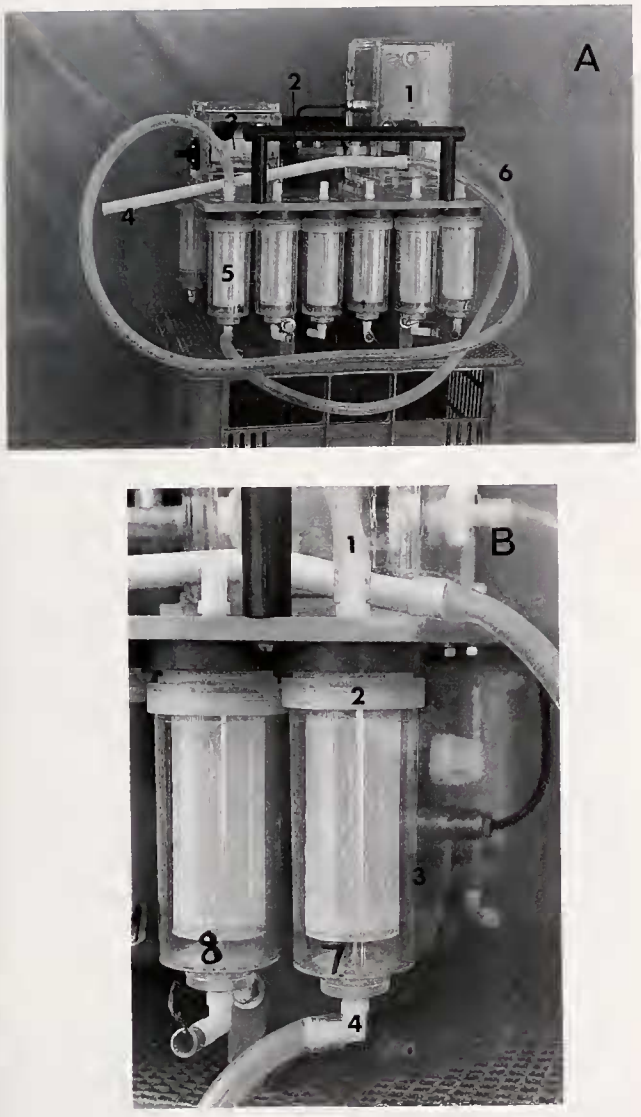

Figure 1. (A) Photograph of apparatus. 1—pump; 2—switch; 3—flow meter; 4—intake hose; 5—filter cartridge assembly; 6—suction hose. (B) Detail of filter cartridge assembly. 1—intake (sample); 2—filter cartridge flange; 3—outer cartridge casing; 4—outlet(suction).

cally, allowing the volume of each sample to be recorded. Power was supplied by two parallel 12-V gel-cell batteries. Batteries, pump, flow meter, and switch were each housed in separate clear acrylic O-ring-sealed housings, giving a maximum safe operating depth of 20 m.

The sampling procedure

The apparatus was used in experiments investigating fertilization rates of eggs from female starfish separated downstream from males at varying distances, and fertilization rates of eggs from female starfish and other echinoderms during natural spawning events. The sampling procedure given here is for experiments involving artificially spawned crown-of-thorns starfish.

The direction and path of the current were determined with fluorescein dye and marked with a plastic-fiber measuring tape. A single male starfish was placed upstream, and the female starfish was placed at a set distance downstream. The sex of starfish was determined by extracting a small amount of gonad with a large-bore syringe. The female starfish was then injected with 20–30 ml of 1×10^{-4} *M* 1-methyl adenine to induce spawning, and the male was injected 10 to 15 min later. A large cloud of fluorescein was released next to the male starfish once spawning was well under way, to mark the passage of the sperm plume. Sampling was started when the female starfish had begun to spawn, but not before the dye cloud had reached the female. Two replicate samples were taken with the female at each prescribed distance, starting from the most distant and proceeding towards the spawning male. Only the female starfish was moved in each trial. Two replicates were always taken with the male and female starfish adjacent to one another (0 m), as a control to establish maximum potential fertilization rates for each experimental pairing. The last pair of samples were taken with the female starfish 8 m upstream from the male, as a sperm-free control. The sperm-free control samples provided a means to ensure that fertilization rates observed in the trial were not due to extraneous sperm from naturally spawning starfish in the area. New animals were obtained for each trial. On completion of the experiment, the apparatus was brought to the surface and transported back to the research vessel, where the eggs were decanted from each filter cartridge and fixed immediately in 10% seawater formalin.

Samples were taken holding the intake of the suction hose approximately 15 cm above the spawning female starfish. Although eggs did accumulate on the surface of the starfish, currents during the experiments (0.07–0.25 $m \cdot s^{-1}$) were sufficient to advect the eggs into the water column. Before sampling at a prescribed distance, the pump, flow meter, and sampling hoses were flushed with water to remove eggs and water from the previous sampling point. The eggs on the surface of the female starfish were shaken free, and sampling was not commenced until a new series of eggs had begun to leave the body surface of the starfish. Current speed was recorded at the site immediately before and after each trial by releasing neutrally buoyant markers and timing their travel along the tape.

Sources of bias

The primary sources of possible bias in the sampling procedure were that (a) variability in the quantity of water sampled through the filter cartridge might influence the fertilization rate and (b) the fertilization rate might in-

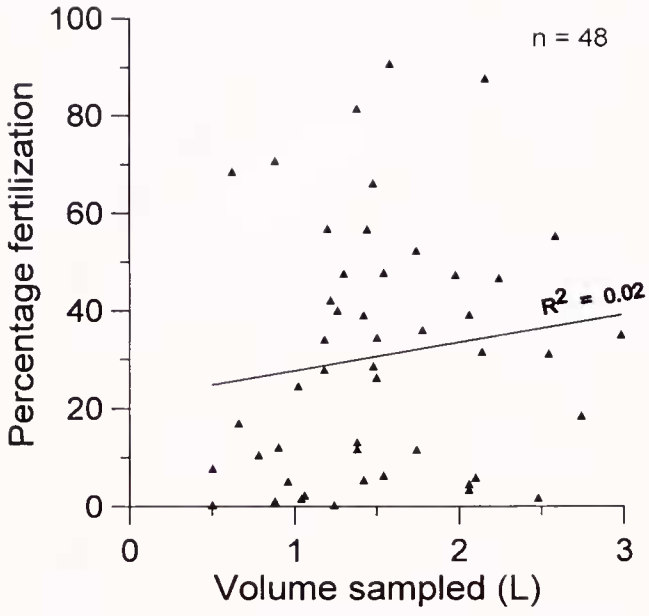

Figure 2. Percentage fertilization at 32 m plotted against volume, V, of water sampled. Regression equation: fertilization = $5.74V + 21.94$ ($R^2 = 0.02$, $n = 48$).

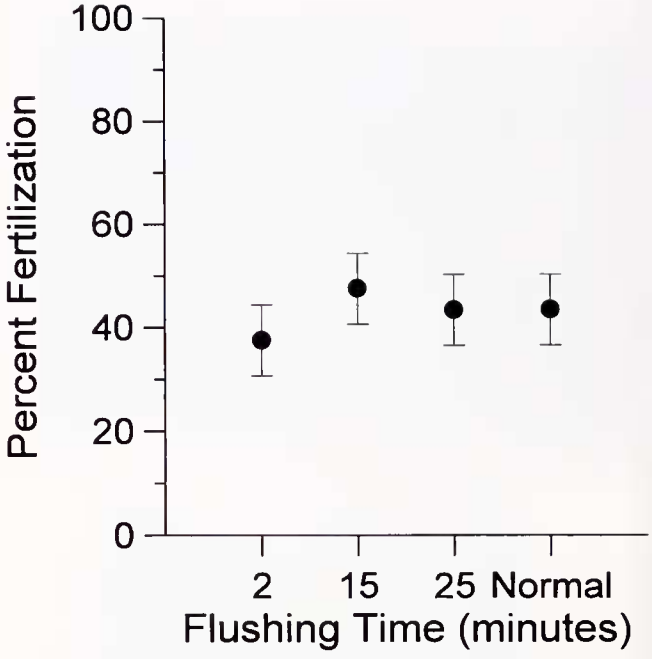

Figure 3. Mean percentage fertilization at 32 m for four flushing treatments (normal and 2, 15, and 25 min), with 95% confidence limits.

crease as a result of the eggs remaining in the filter cartridges at constant sperm concentrations for varying periods of time (under natural conditions it is assumed that sperm continue to decrease in concentration). Both of these sources of bias relate to the possibility of greater sperm exposure for eggs held within filter cartridges than for eggs during the normal dispersal process.

To assess the influence of variable quantities of water passing through the filter cartridges, data from male/female pairs separated by a distance of 32 m were analyzed by linear regression. In this analysis the quantity of water sampled was the independent variable and fertilization rate was the dependent. A second series of experimental trials was carried out to determine the effect of retention time of eggs within the filter cartridge on fertilization rate. In each trial, eight samples of eggs were taken from a single female starfish that was held 32 m downstream from a single spawning male starfish. Two samples were flushed with 4 l of sperm-free water at each of three time intervals (2, 15, and 25 min), and the remaining two samples were processed in the normal manner, remaining in the filter cartridges for about 1 to 2 h before decanting and fixation. The sperm-free water was held in a large double-layered plastic bag beside the apparatus. A further two samples were taken with the female adjacent to the male as an indication of maximum fertilization, and two samples were taken 8 m upstream from the spawning male as a sperm-free control. The eggs were decanted in the normal way. A simple nested ANOVA was used to test for the effect of time prior to flushing on the fertilization rate. The main effect was TIME with four treatments (normal and 2, 15, and 25 min). The nested term was the experimental trial, included to account for variability among experimental trials and differences in fertilization rates among animal pairs.

Results

No relationship was found between the fertilization rate and the quantity of water passed through the filter cartridges during sampling ($R^2 = 0.02$, Fig. 2). The quantity of water sampled through the cartridges varied between 0.5 and 3.0 l, with an average of 1.5 l.

No significant differences in fertilization rate were found between the four flushing treatments (normal and 2, 15, and 25 min); Fig. 3, Table I, ANOVA df = 3, 20; $p > 0.95$). The mean fertilization rate for the 2-min flush treatment

Table I

ANOVA table for the experiment to determine whether the time eggs were held in the filter cartridge affected fertilization rate

Source	Degrees of freedom	Mean square	F	$p > f$
TIME	3	168.18	0.12	0.95
TRIALS	16	1384.33	12.79	0.0001
ERROR	20	108.28		

Four treatments within TIME (normal and 2, 15, and 25 min), and 10 TRIALS nested within the main effect TIME.

(37%) was slightly lower than the fertilization rate observed for the 15 min, 25 min, and normal treatments (48, 44, and 45%), although not significantly so (Fig. 3). The results indicate that most of the fertilization takes place within the first 2 min, and that further periods of confinement with sperm prior to flushing do not increase measured levels of fertilization appreciably. The "normal" procedure of decanting eggs from the filter cartridge upon return to the research vessel does not result in a significant increase in fertilization rate. Levels of fertilization recorded at 8 m upstream from the spawning male were zero; those for adjacent animals ranged from 70 to 90%.

Discussion

The results of fertilization rate experiments presented here agree broadly with those presented elsewhere (Babcock and Mundy, 1992; Babcock *et al.* 1994), with high rates of fertilization for *Acanthaster planci* eggs being recorded at large distances downstream from spawning males. The technique of pumping water samples through filter cartridges worked as expected, with no detectable fertilization upstream from spawning males and high levels of fertilization for adjacent animals. This demonstrates that there is little inhibition of fertilization—if any—as a result of the sampling technique, and no contamination of samples by sperm that might have leaked in from other sampling areas (the samples upstream had no measurable fertilization). The absence of fertilized eggs in the upstream samples also indicates that fertilization membranes were not formed as a mechanical artifact of shear in the tubes.

This apparatus represents a significant improvement on techniques used previously to obtain similar measurements in that it does not rely on passive dispersal of sperm through a porous container (*e.g.*, Levitan *et al.*, 1992). Such containers do allow sperm to penetrate, but they may well reduce flow and exchange of water with the surrounding sea. This reduction would have an effect on fertilization rates. Furthermore, our technique does not rely on preloading of containers with eggs and so can be used in natural spawning situations. Unlike syringe sampling (*e.g.*, Pennington, 1985), it can handle large volumes and is therefore able to sample much more dilute concentrations of eggs.

Neither variable amounts of water pumped through the cartridges (representing varying numbers of sperm pumped past eggs) nor varying periods of retention of sperm with samples in the cartridges introduced significant levels of bias into the results of the sampling. Our method can be used to obtain results with a higher level of accuracy and a greater degree of flexibility than have been previously available for measurements of *in situ* fertilization rates.

Many of the features of the apparatus would lend themselves to applications in other fields, such as the study of micro-scale distribution patterns of planktonic organisms, especially demersal forms. With minor modifications to buoyancy, the equipment could also be adapted to open-water situations—for example, sampling of visible plankton patches over small spatial scales (*e.g.*, Wolanski and Hamner, 1988).

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